

16.16.10

AOAC Official Method 990.10 Mammalian Feces in Grain Products Alkaline Phosphatase Detection Method First Action 1990 Final Action 1994

(Applicable to brown rice cream, oat bran, grits, semolina, pasta flour, farina, barley plus, and corn meal.)

Results of the interlaboratory study supporting acceptance of the method:

Brown rice cream: $s_r = 4.3$; $s_R = 5.3$; $RSD_r = 17.8\%$; $RSD_R = 22.0\%$

Oat bran: $s_r = 5.5$; $s_R = 7.8$; $RSD_r = 25.2\%$; $RSD_R = 36.0\%$

Grits: $s_r = 3.4$; $s_R = 5.6$; $RSD_r = 15.1\%$; $RSD_R = 25.2\%$

Semolina: $s_r = 3.6$; $s_R = 4.7$; $RSD_r = 14.4\%$; $RSD_R = 18.7\%$

Pasta flour: $s_r = 5.5$; $s_R = 5.5$; $RSD_r = 22.0\%$; $RSD_R = 22.0\%$

Farina: $s_r = 2.3$; $s_R = 4.4$; $RSD_r = 9.2\%$; $RSD_R = 18.1\%$

Barley plus: $s_r = 4.1$; $s_R = 6.1$; $RSD_r = 17.5\%$; $RSD_R = 26.0\%$

A. Principle

Intestinal tract of most mammals contains alkaline phosphatase enzyme. Enzyme at test pH and temperature splits phosphate radicals from substrate/pH indicator phenolphthalein diphosphate to produce light pink to red-purple color from free phenolphthalein.

B. Apparatus

(a) *Hot water bath*.—Maintained at $42 \pm 1^\circ\text{C}$.

(b) *Hot plate stirrer and stirring bar*.—See **945.75B(n)** (see 16.1.01). 41 mm ovoid stirring bar is recommended.

(c) *Petri dishes*.—Plastic, disposable, or glass; 150 × 20 or 150 × 15 mm.

(d) *Weighing boats*.—8.1 × 8.1 × 1.9 cm, 100 mL capacity, or approximate equivalent size.

C. Reagents

(a) *Magnesium chloride solution*.—Dissolve 0.203 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ in water and dilute to 500 mL with water. Solution has indefinite shelf life.

(b) *Stock test reagent*.—Dissolve 19.0 g borax ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) and 6.28 g anhydrous Na_2CO_3 in 1 L H_2O with stirring. Add 0.94 g phenolphthalein diphosphate (Sigma P 9875, or equivalent), and stir while adding 2 mL MgCl_2 solution. Preparation is stable ca 4 months at room temperature. Solution should be colorless and ca pH 9.5. Discard if not colorless. Degraded phenolphthalein diphosphate produces pink color in reagent. Store phenolphthalein diphosphate in desiccator below 0°C .

(c) *Liquid test agar*.—Prepare fresh daily and maintain at 42°C ; 150 mL per plate is needed. Measure equal volume of stock test reagent, (b) (half of total test agar volume needed), and of water into separate beakers of appropriate size. Beaker for water must be large enough to accommodate 2 times the volume of water. Reserve stock test reagent. Place beaker of water on hot plate stirrer, add stirring bar, and with rapid stirring, add sufficient agar to water to yield 2% agar solution (1.5 g agar/75 mL H_2O). Continue stirring, and heat to boil (watch for foam-over). Place cover glass on beaker to prevent heat loss. When agar begins to foam, add reserved stock test reagent, pouring reagent down side of beaker to prevent agar from coming out of solution. Stir rapidly over heat ca 1 min.

D. Determination

Weigh 10 g from each well-mixed test product into weighing boat. For each 10 g test portion, use number of plates appropriate for product/feces visibility per product as follows: one plate for corn meal, 2 plates each for grits and semolina flour, and 3 plates each for oat bran, brown rice cream, pasta flour, farina, and barley plus. Cool boiled test agar by placing beaker in larger beaker of cold water. Continually stir test agar and check temperature until solution is 42°C . Pour test agar into Petri dish, ca 150 mL per dish. Immediately distribute monolayer of product onto surface of test agar by gently tapping weighing boat; hold boat so that product flows over 1 side, not from a corner, tilting and moving boat above agar surface as product flows. Let product become wet with test agar before adding another layer. Distribute test portion evenly among appropriate number of Petri dishes. Continue in this manner until entire 10 g test portion has been added. Distribution time should be ca 1 min/10 g test portion. Separation of product and fecal particles is best while test agar is hot. Multiple test portions can be added to respective dishes one at a time, or a little of each test portion to its respective dish sequentially, until all of each test portion has been distributed. If clumping is observed while adding test portion, gently swirl plate before continuing. Swirl only when clumps form.

Let test agar gel with lids off Petri dishes (requires 20 min). Agar is gelled when no agar flows when dish is slightly tipped. (*Note:* Take care not to disturb dispersed material in liquid test agar. If particles are moved, color concentration around particles will be diffused and positive spots will be missed.) An electric fan, blowing gently on Petri dishes, may be used to hasten cooling and gelling. When agar is firm, check for pink to red-purple spots, viewing plate against white background. Color of positive spots should be same as that seen in positive control plate. (Be careful not to count spots that are reddish brown or that have another added color. Extra colors are dyes that leach from grain products themselves.) Examine both top and bottom surfaces of each plate so that line of sight is perpendicular to plate while plate is held at 45° angle to white background on table, with light source directed onto plate from above. View plates from side as well as top and bottom. Mark positive spots on lid of dish, using grease pencil, and mark lid and bottom together, using waterproof marker, so that lid can always be placed in same position.

Incubate Petri dishes 10 min in 42°C water bath. Submerge plates in bath just enough to cover agar level in dishes. When several dishes are incubated at one time, place plates in water in pairs, staggering times so that reading delays are avoided and small, rapidly diffusing pink spots are not missed. Remove plates from water bath after 10 min. Wipe inside of lid to remove fog and hold lid so that bottom edge of lid is 2–3 mm above top edge of Petri dish base while plate is read. Mark spots as before. Replace lid and repeat 10 min incubation 2 times, marking additional pink spots on Petri dish lid after each incubation period. Tally and record number of spots as fecal particles per 10 g test portion. Spots that appear and then are not seen on subsequent checks and spots that are seen on bottom of Petri dish with product are to be counted in tally.

E. Positive Control for Feces and Test Agar Medium

Scatter some ground, known rodent feces on Petri dish of liquid test agar in place of food and continue with method. One control plate is needed for each batch of test agar prepared.

F. Response

Amount, intensity, and range of color (light pink to red-purple) observed will vary depending on size of fecal particles, species, and diet of animal. Particles as small as 250 μ m can be identified.

Reference: *JAOAC* **72**, 766(1989).